Association of type and location of BRCA1 and BRCA2 mutations with risk of breast and ovarian cancer

Published in final edited form as:

JAMA. 2015 April 7; 313(13): 1347–1361. doi:10.1001/jama.2014.5985.

Association of Type and Location of *BRCA1* and *BRCA2* Mutations With Risk of Breast and Ovarian Cancer

Timothy R. Rebbeck, PhD, **Nandita Mitra, PhD**, **Fei Wan, MS**, **Olga M. Sinilnikova, PhD**[†], **Sue Healey**, **Lesley McGuffog**, **Sylvie Mazoyer, PhD**, **Georgia Chenevix-Trench, PhD**, **Douglas F. Easton, PhD**, **Antonis C. Antoniou, PhD**, **Katherine L. Nathanson, MD**, and **the CIMBA Consortium**

Abramson Cancer Center, Perelman School of Medicine at the University of Pennsylvania, Philadelphia (Rebbeck, Nathanson); Center for Clinical Epidemiology and Biostatistics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia (Rebbeck, Mitra, Wan); Centre de Recherche en Cancérologie de Lyon, UMR Inserm, Centre Léon Bérard, Lyon, France (Sinilnikova, Mazoyer); Department of Genetics and Computational Biology, Queensland Institute of Medical Research, Brisbane, Australia (Healey, Chenevix-Trench); Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom (McGuffog, Easton, Antoniou); Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia (Nathanson)

Abstract

IMPORTANCE—Limited information about the relationship between specific mutations in *BRCA1* or *BRCA2* (*BRCA1/2*) and cancer risk exists.

OBJECTIVE—To identify mutation-specific cancer risks for carriers of *BRCA1/2*.

DESIGN, SETTING, AND PARTICIPANTS—Observational study of women who were ascertained between 1937 and 2011 (median, 1999) and found to carry disease-associated *BRCA1* or *BRCA2* mutations. The international sample comprised 19 581 carriers of *BRCA1* mutations and 11 900 carriers of *BRCA2* mutations from 55 centers in 33 countries on 6 continents. We estimated hazard ratios for breast and ovarian cancer based on mutation type, function, and nucleotide position. We also estimated RHR, the ratio of breast vs ovarian cancer hazard ratios. A value of RHR greater than 1 indicated elevated breast cancer risk; a value of RHR less than 1 indicated elevated ovarian cancer risk.

EXPOSURES—Mutations of *BRCA1* or *BRCA2*.

MAIN OUTCOMES AND MEASURES—Breast and ovarian cancer risks.

Corresponding Author: Timothy R. Rebbeck, PhD, Department of Biostatistics and Epidemiology, University of Pennsylvania Perelman School of Medicine, 217 Blockley Hall, 423 Guardian Dr, Philadelphia, PA 19104-6021 (rebbeck@upenn.edu).
[†]Deceased.

Role of the Funder/Sponsor: The funders and sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Information: Funding and acknowledgments are presented in eAppendix 2 and 3 in the Supplement.

RESULTS—Among *BRCA1* mutation carriers, 9052 women (46%) were diagnosed with breast cancer, 2317 (12%) with ovarian cancer, 1041 (5%) with breast and ovarian cancer, and 7171 (37%) without cancer. Among *BRCA2* mutation carriers, 6180 women (52%) were diagnosed with breast cancer, 682 (6%) with ovarian cancer, 272 (2%) with breast and ovarian cancer, and 4766 (40%) without cancer. In *BRCA1*, we identified 3 breast cancer cluster regions (BCCRs) located at c.179 to c.505 (BCCR1; RHR = 1.46; 95% CI, 1.22–1.74; $P = 2 \times 10^{-6}$), c.4328 to c.4945 (BCCR2; RHR = 1.34; 95% CI, 1.01–1.78; $P = .04$), and c. 5261 to c.5563 (BCCR23, RHR = 1.38; 95% CI, 1.22–1.55; $P = 6 \times 10^{-9}$). We also identified an ovarian cancer cluster region (OCCR) from c.1380 to c.4062 (approximately exon 11) with RHR = 0.62 (95% CI, 0.56–0.70; $P = 9 \times 10^{-17}$). In *BRCA2*, we observed multiple BCCRs spanning c.1 to c.596 (BCCR1; RHR = 1.71; 95% CI, 1.06–2.78; $P = .03$), c.772 to c.1806 (BCCR13; RHR = 1.63; 95% CI, 1.10–2.40; $P = .01$), and c.7394 to c.8904 (BCCR2; RHR = 2.31; 95% CI, 1.69–3.16; $P = .00002$). We also identified 3 OCCRs: the first (OCCR1) spanned c.3249 to c.5681 that was adjacent to c.5946delT (6174delT; RHR = 0.51; 95% CI, 0.44–0.60; $P = 6 \times 10^{-17}$). The second OCCR spanned c.6645 to c.7471 (OCCR2; RHR = 0.57; 95% CI, 0.41–0.80; $P = .001$). Mutations conferring nonsense-mediated decay were associated with differential breast or ovarian cancer risks and an earlier age of breast cancer diagnosis for both *BRCA1* and *BRCA2* mutation carriers.

CONCLUSIONS AND RELEVANCE—Breast and ovarian cancer risks varied by type and location of *BRCA1/2* mutations. With appropriate validation, these data may have implications for risk assessment and cancer prevention decision making for carriers of *BRCA1* and *BRCA2* mutations.

Women who have inherited mutations in *BRCA1* (17q21, chromosome 17: base pairs 43,044,294 to 43,125,482) or *BRCA2* (13q12.3, chromosome 13: base pairs 32,315,479 to 32,399,671) have an increased risk of breast and ovarian cancers.[1,2] Little is known about how cancer risks differ by *BRCA1* or *BRCA2* (*BRCA1/2*) mutation type. An “ovarian cancer cluster region” (OCCR) has been reported in both *BRCA1* and *BRCA2* using small sample sets. For *BRCA1*, initially mutations after exon 11 were associated with a 20% lower ovarian cancer risk than mutations in exons 1 through 11.[3] Following that observation, Thompson et al[4] reported an increased risk of ovarian vs breast cancer specifically was associated with mutations in the central portion of exon 11. This association was attributed to both a decrease in breast cancer risk and an increase in ovarian cancer risk in this region. Mutations in exon 11 of *BRCA2* also have been associated with higher ovarian vs breast cancer risk than in other regions of the gene.[5] It was hypothesized that this risk variation might be explained by the failure of *BRCA1/2* exon 11 truncating mutations to trigger nonsense-mediated messenger RNA (mRNA) decay (NMD) because of their extremely large size, contrary to truncating mutations in smaller exons. However, this postulate was not supported by the measures of the relative amounts of mRNA transcript encoded by *BRCA1/2* alleles.[6,7] Murine models of different mutations in *BRCA1/2* also suggest that genotype-phenotype correlations exist.[8,9] To our knowledge, no study has reported whether *BRCA1/2* mutation type is associated with differences in breast and ovarian cancer risk. Thus, we evaluated whether *BRCA1* and *BRCA2* mutation type or location is associated with variation in breast and ovarian cancer risk.

Methods

The Consortium of Investigators of Modifiers of BRCA (CIMBA) initiative is an international collaboration of centers on 6 continents that has collected information about carriers of disease-associated *BRCA1* and *BRCA2* mutations with associated clinical, risk factor, and genetic data.[10] All carriers participated in clinical assessment or research studies at the host institutions after providing informed consent under protocols approved by institutional review boards. For some individuals, ascertainment date reflects the earliest date at which they came to the attention of a clinician or research investigator (eg, when they were first seen in a clinic), even though their research participation, genetic testing, and research data collection may have occurred many years later. Fifty-five centers and multi-center consortia (eTable 1 in the Supplement) in 33 countries submitted deidentified data that met the CIMBA inclusion criteria.[10] Study eligibility criteria included carriage of a disease-associated mutation and clinical data necessary to estimate hazard ratios (ie, cancer diagnosis, ascertainment and follow-up dates). Women were excluded if they carried both a *BRCA1* and *BRCA2* mutation (n = 84).

No races/ethnicities were excluded from this study. All races/ethnicities were included in this report to provide maximal generalizability of results for populations who may be undergoing genetic testing and counseling. All race/ethnicity designations were based on self-report. Race/ethnicity data were collected across the various centers using either fixed categories or open-ended questions.

Mutation Classification

Only carriers with clearly pathogenic *BRCA1/2* mutations were included in this analysis. Pathogenic mutations were defined as (1) mutations generating a premature termination codon, except variants generating a premature termination codon in exon 27 after codon 3010 of *BRCA2*[11]; (2) large in-frame deletions that span 1 or more exons; and (3) deletions of transcription regulatory regions (promoter and/or first exon) expected to cause lack of expression of mutant allele. We also included missense variants considered pathogenic by the Breast Cancer Information Core committee or published variants classified as pathogenic using multifactorial likelihood approaches.[12,13] Mutations are described here using the Human Genome Variation Society nomenclature in which the nucleotide numbering is from the A of the ATG translation initiator codon, and use the c.XXX numbering convention (eAppendix 1 in the Supplement).

Creation of Mutation Groups for Analysis

Mutation Bins—To identify segments across the intronic and exonic regions of the *BRCA1* or *BRCA2* genes associated with different breast vs ovarian cancer risks, we created bins of mutations by base pair location (Figure 1). We divided the genomic regions of both genes to create bins of genomic sequence that contained all deleterious mutations regardless of category or function. Bins were constructed by using an algorithm in which each bin contained approximately equal numbers of participants with bin length defined by distance in base pairs. We excluded large genomic rearrangements from this analysis as those mutations span multiple bins and also undertook a subset analysis with and without missense

mutations. The resulting bins are presented in Figure 2 and eTable 2 in the Supplement for *BRCA1* and Figure 3 and eTable 3 in the Supplement for *BRCA2*.

Mutation Type and Functional Domains—Mutations were grouped by type and function as frame shift, nonsense, missense, splice site, and then by in-frame and out-of-frame. Mutation groups included individuals who carried in-frame deletions, nonsplice out-of-frame deletions, and out-of-frame deletions. Missense mutations in *BRCA1* were grouped into those within the RING[14,15] and BRCT domains.[16–19] Only 17 *BRCA2* carriers (0.1%) had missense mutations classified as pathogenic; these were removed from the analysis because the sample size was too small to provide statistically meaningful inferences. Comparisons also were made of mutations predicted not to lead to NMD vs those that do lead to NMD. Mutations predicted not to cause NMD were defined as those that lead to a stop codon within 50 nucleotides before or within the last exon.[20] In *BRCA1*, a subgroup including premature termination codons before c.297, presumed to allow reinitiation of translation at the AUG at that site, was examined separately.[21] Premature termination codons refer to all mutations leading to a truncated open reading frame. Putative functional domains in *BRCA1* and *BRCA2* were defined using the boundaries in the Pfam database.[22] We also identified reported domains in *BRCA1* or *BRCA2* that are involved in binding putative proteins.

Statistical Analysis

The primary outcomes of interest were diagnosis of ovarian cancer or breast cancer. For ovarian cancer, observations were censored at the earliest of the following outcomes: bilateral risk-reducing salpingo-oophorectomy, death, or having reached the end of follow-up without an ovarian cancer or other censoring event. In women with both breast and ovarian cancer diagnoses, prior breast cancer diagnoses were ignored in the analysis of ovarian cancer. Time to event was computed from birth to age at first ovarian cancer diagnosis or age at censoring. For the primary event of breast cancer, observations were censored at the earliest of the following outcomes: ovarian cancer, risk-reducing salpingo-oophorectomy, risk-reducing mastectomy, death, or having reached the end of follow-up without a cancer or other censoring event. Time to event was computed from birth to age at first cancer diagnosis or age at censoring. To account for intracluster dependence due to multiple individuals from the same family, a robust sandwich variance estimate was specified in Cox proportional hazards models.[23] All analyses were undertaken in *BRCA1* and *BRCA2* mutation carriers separately. The proportional hazards assumption was tested using log(-log) plots and Schoenfeld residuals.

Our analyses assessed the relationship of mutation groups with cancer risk. First, we used mutation bins to evaluate whether there is evidence to support the previous report of an OCCR[3,5] and whether breast cancer cluster regions (BCCRs) may exist. To assess whether specific genomic regions of these genes were associated with greater breast vs ovarian cancer risk, we computed the hazard ratio of breast cancer, the hazard ratio of ovarian cancer, and a statistic RHR, defined as the ratio of breast vs ovarian cancer hazard ratio estimates. Values of RHR greater than 1 indicate elevated breast cancer risk; values of RHR less than 1 indicate elevated ovarian cancer risk. We evaluated bins of mutations across the

span of *BRCA1* or *BRCA2* compared with all other mutations not contained in that bin by fitting a multiple correlated outcomes model stratified by cancer site.[24] This approach allowed us to achieve 2 goals: first, to estimate the correlation between ovarian and breast cancer outcomes within an individual, and second, to provide an estimate of the RHR (estimated via an interaction term between cancer site and mutation bin) with the correct confidence interval using robust sandwich variance estimates to account for the correlation between outcomes within a woman. All analyses were adjusted for birth year and race, stratified by center, and controlled for clustering within family.

Second, we compared each mutation type or functional group against a common reference group. The use of a common reference group allowed us to compare hazard ratio estimates across different mutation classes. For both *BRCA1* and *BRCA2*, we chose exon 11 nonsense mutations as the common reference group. Exon 11 nonsense mutations are common in diverse ethnic backgrounds and have been demonstrated to have the same biological effect, leading to NMD.[25]

Approximate cancer risks to age 70 years for specific mutation classes were derived from the relative risk estimates. For *BRCA1* and *BRCA2*, estimated lifetime breast cancer penetrances were assumed to be 59% and 51% and ovarian cancer penetrance 34% and 11%, respectively.[26] Mutation-specific penetrance estimates were derived using the method presented in eAppendix 2 in the Supplement.

Statistical tests were judged significant based on 2-sided hypothesis tests with $P < .05$. All P values were corrected for multiple hypothesis testing within each table of results by controlling the false discovery rate (FDR) using the method of Benjamini and Hochberg.[27] Analyses were conducted in SAS version 9 (SAS Institute) or R version 2.7.2 (R Foundation for Statistical Computing).

Results

A total of 19 581 female carriers of *BRCA1* mutations and 11 900 carriers of *BRCA2* mutations were eligible for inclusion in this study. Table 1 reports the distribution of dates of ascertainment to the study as well as time from ascertainment to cancer diagnosis or censoring, as used in the survival analysis models reported in this section. Mean age of breast cancer diagnosis was 39.9 years in *BRCA1* mutation carriers and 42.8 years in *BRCA2* mutation carriers. Mean age of ovarian cancer diagnosis was 50.0 years in *BRCA1* mutation carriers and 54.5 years in *BRCA2* mutation carriers. The 3 ovarian cancer cases diagnosed before age 18 years were germ cell tumors and included in the analysis (Table 1). Of note, all analyses also were undertaken excluding these 3 cases and there was no difference in the results. The majority of the sample consisted of white women for both *BRCA1* and *BRCA2* mutation carriers: 92% to 93% white, including 8% to 9% Jewish women. Both *BRCA1* and *BRCA2* mutation carriers had a median parity of 2.0 live births and age at menarche of 13 years. Median age at menopause was 44 years in *BRCA1* and 46 years in *BRCA2* mutation carriers, reflecting in part the use of preventive surgeries.

BRCA1: Breast and Ovarian Cancer Cluster Regions

We observed an OCCR bounded by c.1380 and c.4062 (Figure 2), suggesting a relative decrease in breast relative to ovarian cancer risk (RHR = 0.62; 95% CI, 0.56–0.70; FDR-corrected $P = 9 \times 10^{-17}$). This estimate was obtained by considering all mutations across multiple bins spanning the OCCR. The OCCR is explained by both a relative decrease in breast cancer risk and a relative increase in ovarian cancer risk (eTable 2 in the Supplement), which was statistically significant in bins 9, 11–13, 15–16, and 23 (Figure 2). The OCCR extends further 53 of the previously reported OCCR, which was defined by the interval c.2282 to c.4071.[3] The OCCR is entirely contained within exon 11 (c.670-c.4096) with bins 6 and 23 being approximately coincident with the boundaries of the exon.

We also observed a relative increase in breast cancer risk and a relative decrease in ovarian cancer risk for mutations occurring in the 53 and 33 regions of *BRCA1*, potentially defining 2 BCCRs (Figure 2). BCCR1 mutations within bins 4–5 (c.179-c.505) were associated with excess risks of breast vs ovarian cancer (eTable 2 in the Supplement) and lie within the 33 region of the RING domain (c.72-c.192). Mutations in the BCCR1 were associated with a relative increase in breast cancer risk relative to ovarian cancer risk (RHR = 1.46; 95% CI, 1.22–1.74; FDR-corrected $P = 2 \times 10^{-6}$). When all mutations in the RING domain were considered together as compared with all others, they were associated with a significant increase in breast cancer risk (HR = 1.13; 95% CI, 1.02–1.26) and a significant decrease in ovarian cancer risk (HR = 0.81; 95% CI, 0.67–0.97). Bin 2, which contains only the founder mutation *BRCA1* c.68_69delAG (185delAG), did not provide statistically significant evidence for elevated breast vs ovarian cancer risks, suggesting that this mutation is associated with relatively equivalent risks of both cancers.

Mutations in bins 26 and 29–30 in the 33 region of *BRCA1* also provided evidence for additional BCCRs. BCCR2 was associated with an increase in breast cancer relative to ovarian cancer risk (RHR = 1.34; 95% CI, 1.01–1.78; $P = .04$) bounded by c.4328 and c. 4945. The second segment of this BCCR (denoted BCCR23) includes the BRCT domains (c. 4926-c.5169 and c.5268-c.5526) and was associated with a relative excess of breast vs ovarian cancers (RHR = 1.38; 95% CI, 1.22–1.55; $P = 6 \times 10^{-9}$) (Figure 2). In the BRCT domains, the preponderance of mutations was missense, not expected to trigger NMD. This region also includes bin 29, which contains only the *BRCA1* c.5266dupC (5382insC) mutation, which also is not predicted to lead to NMD as it introduces a premature termination codon in the last exon.[6] In bin 30, *BRCA1* c.5277 + 1G>A, a common splice site mutation in the Netherlands, is observed and not expected to lead to NMD.

We compared breast and ovarian cancer risks between women who had a mutation in a specified functional domain compared with all other women who did not have mutations in that domain. Mutations in the RING domain were associated with higher breast cancer risks and nonsignificant lower ovarian cancer risks than other mutations. These results are consistent with the colocation of the BCCR1 (Figure 2) and RING domain. Mutations in the BRCT domains were associated with higher breast cancer risk. When analyses were limited to mutations conferring NMD, breast cancer risk became significantly associated with mutations in the coiled coil domain.

BRCA1: Risks by Category and Function

We observed variability in breast and ovarian cancer risks by mutation class (Table 2 and Table 3). For *BRCA1*-associated breast cancer, most risk groups were associated with higher breast cancer risk than the exon 11 nonsense mutation reference group. This result is consistent with the data shown in Figure 2 and eTable 2 in the Supplement as it is similar in location with the OCCR. Groups with elevated breast cancer risk include all mutations leading to NMD (group 1), all premature termination codon mutations except for exon 11 nonsense mutations (group 2), frame shift and nonsense mutations occurring 53 of c.297 that are predicted to lead to NMD and reinitiation (group 3), nonpremature termination codon mutations (group 4), all founder mutations (group 5) and the founder mutation c.5266dup C (group 5b), missense mutations (group 6) and missense mutations in the RING domain (group 6a), missense and in-frame deletions (group 7), all in-frame deletions (group 8), and premature termination codon mutations not leading to NMD (group 9). The majority of the last group is comprised of c.5266dupC (83%). For *BRCA1*-associated ovarian cancer (Table 2), mutations associated with significantly lower ovarian cancer risks compared with the reference group included mutations 53 of c.297 (group 3), nonpremature termination codons (group 4), founder mutations (group 5, 5a, 5b), missense mutations (group 6, 6a), missense and in-frame deletions (group 7), and premature termination codons not leading to NMD (group 9).

When comparing mean age differences among women with or without a specific mutation category or function, we found small but statistically significant differences. In *BRCA1*, exon 11 mutations were associated with earlier ages at breast and ovarian cancer diagnosis. Mutations conferring NMD or premature termination codon were associated with a later age at breast cancer diagnosis. Conversely, an earlier age at breast cancer diagnosis was associated with nonpremature termination codon mutations and the founder mutations (Table 3).

BRCA2: Breast and Ovarian Cancer Cluster Regions

We observed an OCCR (OCCR1) bounded by c.3249 and c.5681, containing c.5946delT (6174delT), with statistically significant evidence for a relatively higher ovarian cancer vs breast cancer risk among carriers of mutations in bins 6–9 and 11 (Figure 3). OCCR1 is explained by both a relative increase in ovarian cancer risk and a relative decrease in breast cancer risk, with an increase in ovarian cancer relative to breast cancer risk (RHR = 0.51; 95% CI, 0.44–0.60; $P = 6 \times 10^{-17}$) (eTable 3 in the Supplement). The putative OCCR1 lies within the previously reported OCCR[3] and approximately colocalized with the BRC repeats within exon 11.[17,18,28] A second putative OCCR (OCCR2) outside of the original OCCR boundaries also was observed defined by bin 14 (c.6645-c.7471). OCCR2 was associated with an increase in ovarian cancer relative to breast cancer risk (RHR = 0.57; 95% CI, 0.41–0.80; $P = .001$).

We also observed a relative increase in breast cancer risk and a relative decrease in ovarian cancer risk for mutations occurring in the 53 and 33 regions of *BRCA2*, potentially defining multiple BCCRs (ie, BCCR1, BCCR13, and BCCR2) (Figure 3). These 3 regions were associated with relatively increased breast cancer risk relative to ovarian cancer risk with

RHR = 1.71 (95% CI, 1.06–2.78; *P* = .03), RHR = 1.63 (95% CI, 1.10–2.40; *P* = .01), and RHR = 2.31 (95% CI, 1.69–3.16; *P* = .00002), respectively. These regions were associated with both increased breast cancer risk and decreased ovarian cancer risk (eTable 3 in the Supplement).

We also observed small but statistically significant differences in the mean age at breast cancer diagnosis associated with some of these regions. The mean age was greater for mutations in OCCR vs mutations not in OCCR (45.0 vs 43.9 years, *P* < .001; mean difference: 1.17; 95% CI, 0.65 to 1.69), lower for mutations in BCCR1 vs mutations not in BCCR1 (42.6 vs 44.3 years; *P* = .004; mean difference: −1.66, 95% CI, −2.80 to −0.53), and lower for mutations in BCCR2 vs mutations not in BCCR2 (43.5 vs 44.3 years, *P* = .04; mean difference: −0.80, 95% CI, −1.55 to −0.05).

To complement the prior set of analyses, we also present associations of breast and ovarian cancers among groups of *BRCA2* mutation carriers defined by known DNA binding domains (Table 4). Mutations in the BRC repeats were associated with lower breast cancer risks and higher ovarian cancer risks than those mutations not occurring in the BRC repeats consistent with their colocation with the OCCR1 (Figure 3).

BRCA2: Risks by Category and Function

For *BRCA2*-associated cancer, the reference exon 11 mutation group was associated with decreased breast cancer risk compared with most other mutation classes (Table 2), consistent with colocalization with OCCR1. Compared with the reference group, ovarian cancer risks were further reduced among women who carried premature truncation codons (HR = 0.27; 95% CI, 0.11–0.66). We observed an association with earlier age at breast cancer diagnosis with exon 11 mutations and for mutations not conferring NMD (Table 3).

Absolute Risks

To illustrate potential mutation-specific effects on absolute cancer risks, we used the hazard ratio estimates to derive approximate absolute risks and 95% confidence intervals, based on published estimates for the overall risks of breast and ovarian cancer by age 70 years.[26] These estimates are for illustration and do not represent absolute risk estimates that would be required in a genetic counseling setting, as they do not account for noncancer outcomes that may influence a woman's life expectancy, the effects of family history, and nonrandom ascertainment of mutation carriers in this sample and depend on assumptions about the prevalence of different mutation classes in the population. Using the *BRCA1/2* baseline breast and ovarian cancer risks of Antoniou et al,[26] we estimated risks and confidence intervals about these risks (eAppendix 2 in the Supplement). These confidence limits assume that the overall risk for a given individual is provided by our estimates and should not be interpreted as measuring the overall uncertainty in the absolute risk estimates, as shown in Table 5. The overall breast cancer risk for *BRCA1* mutation carriers by age 70 years is 59%, which increases to 69% (95% CI, 56%–83%) in women who carry a missense mutation, Jewish founder mutation, or a mutation that undergoes NMD with reinitiation. The ovarian cancer risk in *BRCA1* mutation carriers by age 70 years is 34% overall but decreases to 26% (95% CI, 10%–43%) among women who carry a founder mutation.

Discussion

We have identified mutations in *BRCA1* or *BRCA2* that are associated with significantly different risks of breast and ovarian cancers. These mutation-specific risks coincide with known or hypothesized functional domains and provide a basis around which accurate risk estimates can be generated for women who have inherited a particular *BRCA1/2* mutation. These results are consistent with prior reports of OCCRs in both *BRCA1* and *BRCA2* that lie in or near exon 11 of both genes.[3,5,29,30] Mutations in exon 11 could produce a partial BRCA1 protein encoded by the known exon 11 splice variant, while the full-length protein is lost by the process of NMD.[6] Murine embryos carrying the exon 11–deleted isoform survive longer than those that are *BRCA1* null, and *BRCA1* that has lost exon 11 appear to retain partial function.[31] Thus, for *BRCA1*, it is biologically plausible that individuals carrying mutations within exon 11 (and the OCCR) may have a different phenotype than other mutations. In *BRCA2*, we have identified OCCRs, coincident with the 8 BRC repeats. Mutations in this region appear to be associated with NMD, which would lead to loss of *BRCA2* expression.[7] However, it is possible that there is persistence of an alternatively spliced variant of *BRCA2*, without exon 11 (as for *BRCA1*), which would represent in-frame mutations. In addition, the *BRCA2* BRC repeats interact with *RAD51*, which has been consistently shown to be a modifier of *BRCA2*-associated breast and ovarian cancer risk.[32] Without the BRC repeats, *BRCA2* might differ in interactions with *RAD51* and lead to genotype-phenotype variation. However, the biological basis of the *BRCA2* OCCR remains speculative, in particular as it does not extend throughout all of exon 11.

In *BRCA2*, several putative BCCRs were defined. The 33 BCCR coincides approximately with mutations occurring in the oligonucleotide binding (OB) fold domains and the tower domain. When examined independently, both of these domains were associated with relatively elevated breast cancer risk and lower ovarian cancer risk. These mutations in *BRCA2* would be predicted to undergo NMD. However, it has been demonstrated experimentally for only a few mutations, leaving the functional basis unknown.[7]

We have also identified a decreased risk of ovarian cancer associated with all types of mutations predicted not to lead to NMD in *BRCA2*; the estimated risk was only significant for all mutations together and those mutations leading to in-frame splice site or frame shift mutations. These mutations all occur after nucleotide 7000 in the C-terminus of *BRCA2*, which includes the DNA binding domains, tower domains, and OB folds.[33] These functional domains are associated with localization of *BRCA2* to sites of double-stranded DNA breaks to accomplish repair.[33] These data suggest that intact protein may be protective when it comes to ovarian cancer risk. However, the number of individuals is small and further replication is needed.

A number of limitations of this research may influence the generalizability and translational potential of this research. Despite the very large sample size, we were not able to investigate some mutation and risk groups with adequate statistical power. Carriers of *BRCA2* mutations composed a smaller sample set; in particular, the number of women with *BRCA2*-associated ovarian cancers was relatively small. Although all women with a documented disease-associated mutation in the CIMBA database were included, some populations use screening

for founder mutations as a primary method of mutation detection, such as for the 3 Ashkenazi Jewish mutations. This testing strategy may lead to underreporting of nonfounder mutations. As such, some bias in the ascertainment of the full spectrum of mutations could have occurred. The ascertainment strategy generally followed clinical and research protocols similar across all centers. However, we did not correct for ascertainment, and thus bias may have affected some variables (eg, age at diagnosis), which should be interpreted with caution. Mutation testing was performed using methods acceptable for clinical practice at each center, which was not uniform across all centers.

The present sample set does not reflect the general population of all mutation carriers but reflects those women who have undergone genetic testing for *BRCA1/2* mutations, a relevant population of inference. We have presented the mutations in terms of category or effect, but these designations are in some cases extrapolated based on experimental evidence for similar mutations. An example is the designation of NMD inferred from mutation location, which is based on experimental validation of only a small proportion of the mutations.[6,7] Similarly, inference of protein truncation based on predicted protein-truncating mutations without experimental verification may lead to erroneous classification.[25] Penetrances that are presented here are limited because other factors that are not accounted for here could influence these estimates. These factors include family history and competing mortality. These risks also depend on knowing the true prevalence of the mutation-specific classes, which is likely to be population-specific.

In addition, the present report of more than 32 000 mutation carriers could include some of those individuals who were included in the 1995 and 1997 articles that originally reported the OCCR.[3] It is not possible at this time to know if any of the 32 families carrying *BRCA1* mutations or 25 families carrying *BRCA2* originally reported also are included in the present sample. However, it is highly unlikely that the small sample of individuals represented in the original reports would outweigh a potential null effect among the more than 32 000 individuals studied here.

This study is the first step in defining differences in risk associated with location and type of *BRCA1* and *BRCA2* mutations. Pending additional mechanistic insights into the observed associations, knowledge of mutation-specific risks could provide important information for clinical risk assessment among *BRCA1/2* mutation carriers, but further systematic studies will be required to determine the absolute cancer risks associated with different mutations. It is yet to be determined what level of absolute risk change will influence decision making among carriers of *BRCA1/2* mutations. Additional research will be required to better understand what level of risk difference will change decision making and standards of care, such as preventive surgery,[34] for carriers of *BRCA1* and *BRCA2* mutations.

Conclusions

Breast and ovarian cancer risks varied by type and location of *BRCA1/2* mutations. With appropriate validation, these data may have implications for risk assessment and cancer prevention decision making among carriers of *BRCA1* and *BRCA2* mutations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding/Support: Dr Antoniou and the CIMBA data management are funded by Cancer Research UK. Dr Easton is a Principal Research Fellow of Cancer Research UK. Dr Rebbeck is supported by R01-CA083855, R01-CA102776, and P50-CA083638 from the National Institutes of Health (NIH). Dr Rebbeck, Dr Nathanson, Ms Friebel, and Dr Domchek are supported by the Basser Research Center at the University of Pennsylvania. Dr Nathanson is supported by the Breast Cancer Research Foundation. Drs Domchek and Nathanson are supported by the Rooney Family Foundation. Dr Poppe is supported by R01-CA112520 from NIH. Cancer Research UK provided financial support for this work. Dr Antoniou is a Senior Cancer Research UK Cancer Research Fellow. Dr Phillips is a National Breast Cancer Foundation (Australia) Practitioner Fellow.

Appendix

Authors/CIMBA Consortium members include the byline authors as well as the following individuals: Yael Laitman, MSC; Anya Kushnir, MSC; Shani Paluch-Shimon, MD; Raanan Berger, MD; Jamal Zidan, MD; Eitan Friedman, MD, PhD; Hans Ehrencrona, MD; Marie Stenmark-Askmalm, MD; Zakaria Einbeigi, MD; Niklas Loman, MD; Katja Harbst, PhD; Johanna Rantala, PhD; Beatrice Melin, MD, PhD; Dezheng Huo, PhD; Olufunmilayo I. Olopade, MD; Joyce Seldon, MSGC; Patricia A. Ganz, MD; Robert L. Nussbaum, MD; Salina B. Chan, BS; Kunle Odunsi, MD, PhD; Simon A. Gayther, PhD; Susan M. Domchek, MD; Banu K. Arun, MD; Karen H. Lu, MD; Gillian Mitchell, MD; Beth Y. Karlan, MD; Christine Walsh, MD; Jenny Lester, MPH; Andrew K. Godwin, PhD; Harsh Pathak, PhD; Eric Ross, PhD; Mary B. Daly, MD, PhD; Alice S. Whittemore, PhD; Esther M. John, PhD, MSPH; Alexander Miron, PhD; Mary Beth Terry, PhD; Wendy K. Chung, MD, PhD; David E. Goldgar, PhD; Saundra S. Buys, MD; Ramūnas Janavičius, MD, PhD; Laima Tihomirova, MD; Nadine Tung, MD; Cecilia M. Dorfling, PhD; Elizabeth J. van Rensburg, PhD; Linda Steele, BS; Susan L. Neuhausen, PhD; Yuan Chun Ding, PhD; Bent Ejlertsen, MD, PhD; Anne-Marie Gerdes, MD; Thomas v. O. Hansen, MD; Teresa Ramón y Cajal, MD; Ana Osorio, PhD; Javier Benitez, MD; Javier Godino, MD; Maria-Isabel Tejada, PhD; Mercedes Duran, PhD; Jeffrey N. Weitzel, MD; Kristie A Bobolis, MD; Sharon R. Sand, CCRP; Annette Fontaine, MD; Antonella Savarese, MD; Barbara Pasini, MD; Bernard Peissel, MD; Bernardo Bonanni, MD; Daniela Zaffaroni, PhD; Francesca Vignolo-Lutati, PhD; Giulietta Scuvera, MD; Giuseppe Giannini, MD; Loris Bernard, PhD; Maurizio Genuardi, MD; Paolo Radice, PhD; Riccardo Dolcetti, MD; Siranoush Manoukian, MD; Valeria Pensotti, PhD; Viviana Gismondi, PhD; Drakoulis Yannoukakos, PhD; Florentia Fostira, PhD; Judy Garber, MD, MPH; Diana Torres, MD; Muhammad Usman Rashid, MD; Ute Hamann, PhD; Susan Peock, MS; Debra Frost, MS; Radka Platte, BS; D. Gareth Evans, MD; Rosalind Eeles, PhD; Rosemarie Davidson, PhD; Diana Eccles, MD; Trevor Cole, MD; Jackie Cook, MD; Carole Brewer, MD; Shirley Hodgson, MD; Patrick J. Morrison, MD, DSc; Lisa Walker, MS; Mary E. Porteous, MD; M. John Kennedy, PhD; Louise Izatt, MBBChir, PhD; Julian Adlard, MD; Alan Donaldson, MD; Steve Ellis, MSc; Priyanka Sharma, PhD; Rita Katharina Schmutzler, MD; Barbara Wappenschmidt, MD; Alexandra Becker, MD; Kerstin Rhiem, MD; Eric Hahnen, MD; Christoph Engel, MD; Alfons Meindl, MD; Stefanie Engert, MD; Nina Ditsch, MD; Norbert Arnold, MD; Hans Jörg Plendl, MD;

Christoph Mundhenke, MD; Dieter Niederacher, MD; Markus Fleisch, MD; Christian Sutter, PhD; C. R. Bartram, MD; Nicola Dikow, MD; Shan Wang-Gohrke, MD; Dorothea Gadzicki, MD; Doris Steinemann, MD; Karin Kast, MD; Marit Beer, MD; Raymonda Varon-Mateeva, MD; Andrea Gehrig, MD; Bernhard H. Weber, MD; Dominique Stoppa-Lyonnet, PhD; Olga M. Sinilnikova, PhD; Sylvie Mazoyer, PhD; Claude Houdayer, PhD; Muriel Belotti, PhD; Marion Gauthier-Villars, PhD; Francesca Damiola, PhD; Nadia Boutry-Kryza, PhD; Christine Lasset, PhD; Hagay Sobol, PhD; Jean-Philippe Peyrat, PhD; Danièle Muller, PhD; Jean-Pierre Fricker, MD; Marie-Agnès Collonge-Rame, PhD; Isabelle Mortemousque, PhD; Catherine Nogues, PhD; Etienne Rouleau, PhD; Claudine Isaacs, MD; Anne De Paepe, MD; Bruce Poppe, MD; Kathleen Claes, PhD; Kim De Leeneer, MD; Marion Piedmonte, MA; Gustavo Rodriguez, MD; Katie Wakely, MD; John Boggess, MD; Stephanie V. Blank, MD; Jack Basil, MD; Masoud Azodi, MD; Kelly-Anne Phillips, MD; Trinidad Caldes, MD; Miguel de la Hoya, PhD; Atocha Romero, PhD; Heli Nevanlinna, PhD; Kristiina Aittomäki, MD; Annemarie H. van der Hout, PhD; Frans B. L. Hogervorst, PhD; Senno Verhoef, PhD; J. Margriet Collée, PhD; Caroline Seynaeve, MD, PhD; Jan C. Oosterwijk, MD, PhD; Johannes J. P. Gille, PhD; Juul T. Wijnen, PhD; Encarna B. Gómez Garcia, MD; Carolien M. Kets, MD; Margreet G. E. M. Ausems, MD; Cora M. Aalfs, MD; Peter Devilee, MD; Arjen R. Mensenkamp, PhD; Ava Kwong, MD; Edith Olah, PhD, DSc; Janos Papp, PhD; Orland Diez, PhD; Conxi Lazaro, PhD; Esther Darder, PhD; Ignacio Blanco, MD; Mónica Salinas, MS; Anna Jakubowska, PhD; Jan Lubinski, MD; Jacek Gronwald, PhD; Katarzyna Jaworska-Bieniek, PhD; Katarzyna Durda, PhD; Grzegorz Sukiennicki, PhD; Tomasz Huzarski, PhD; Tomasz Byrski, PhD; Cezary Cybulski, PhD; Aleksandra Toloczko-Grabarek, PhD; Elżbieta Złowocka-Perlowska, PhD; Janusz Menkiszak, ND; Adalgeir Arason, ND; Rosa B. Barkardottir, ND; Jacques Simard, PhD; Rachel Laframboise, MD; Marco Montagna, PhD; Simona Agata, PhD; Elisa Alducci, PhD; Ana Peixoto, PhD; Manuel R. Teixeira, MD, PhD; Amanda B. Spurdle, PhD; Min Hyuk Lee, MD; Sue K. Park, MD; Sung-Won Kim, MD, PhD; Tara M. Friebel, MPH; Fergus J. Couch, PhD; Noralane M. Lindor, MD; Vernon S. Pankratz, PhD; Lucia Guidugli, PhD; Xianshu Wang, PhD; Marc Tischkowitz, PhD; Lenka Foretova, MD; Joseph Vijai, MD; Kenneth Offit, MD; Mark Robson, MD; Rohini Rau-Murthy, MD; Noah Kauff, MD; Anneliese Fink-Retter, MD; Christian F. Singer, MD; Christine Rappaport, MD; Daphne Gschwantler-Kaulich, MD; Georg Pfeiler, MD; Muy-Kheng Tea, MD; Andreas Berger, MD; Mark H. Greene, MD; Phuong L. Mai, PhD; Evgeny N. Imyanitov, MD; Amanda Ewart Toland, PhD; Leigha Senter, MD; Anders Bojesen, PhD; Inge Sokilde Pedersen, PhD; Anne-Bine Skytte, PhD; Lone Sunde, PhD; Mads Thomassen, PhD; Sanne Traasdahl Moeller, PhD; Torben A. Kruse, PhD; Uffe Birk Jensen, PhD; Maria Adelaide Caligo, MD; Paolo Aretini, MD; Soo-Hwang Teo, PhD; Christina G. Selkirk, MS, CCRP; Peter J. Hulick, MD, MMSc; Irene Andrulis.

Affiliations of Authors/CIMBA Consortium

Abramson Cancer Center, Perelman School of Medicine at the University of Pennsylvania, Philadelphia (Domchek); Center for Clinical Epidemiology and Biostatistics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia (Friebel); Department of Genetics and Computational Biology, Queensland Institute of Medical Research, Brisbane,

Australia (Spurdle); Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom (Peock, Frost, Platte, Ellis); Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia (Domchek); Sheba Medical Center, Tel Aviv, Israel (Laitman, Paluch-Shimon, Friedman); Susanne Levy Gertner Oncogenetics Unit, Sheba Medical Center, Tel Hashomer, Israel (Kushnir); Oncology Institute, Sheba Medical Center, Tel Hashomer, Israel (R. Berger); Oncology Institute, Rivkah Ziv Medical Center Zefat, Israel (Zidan); Department of Oncology, Lund University, Lund, Sweden (Ehrencrona, Loman, Harbst); Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden (Ehrencrona); Division of Clinical Genetics, Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden (Stenmark-Askmalm); Department of Oncology, Sahlgrenska University Hospital, Gothenburg, Sweden (Einbeigi); Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden (Rantala); Department of Radiation Sciences, Oncology, Umeå University, Umeå, Sweden (Melin); Center for Clinical Cancer Genetics and Global Health, University of Chicago Medical Center, Chicago, Illinois (Huo, Olopade); UCLA Schools of Medicine and Public Health, Division of Cancer Prevention and Control Research, Jonsson Comprehensive Cancer Center, Los Angeles, California (Seldon, Ganz); Department of Medicine and Genetics, University of California, San Francisco (Nussbaum); Cancer Risk Program, Helen Diller Family Cancer Center, University of California, San Francisco (Chan, Whittemore); Department of Gynecologic Oncology, Roswell Park Cancer Institute, Buffalo, New York (Odunsi); Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles (Gayther); University of Texas MD Anderson Cancer Center, Houston (Arun, Lu); Division of Cancer Medicine, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia (Mitchell, Phillips); Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Australia (Mitchell, Phillips); Women's Cancer Program at the Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, California (Karlan, Walsh, Lester); Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City (Godwin, Pathak); Fox Chase Cancer Center, Philadelphia, Pennsylvania (Ross); Department of Health Research and Policy, Stanford University School of Medicine, Stanford, California (Daly); Department of Epidemiology, Cancer Prevention Institute of California, Fremont (John); Dana-Farber Cancer Institute, Boston, Massachusetts, (Miron, Garber); Department of Epidemiology, Columbia University, New York, New York (Terry); Departments of Pediatrics and Medicine, Columbia University, New York, New York (Chung); Department of Dermatology, University of Utah School of Medicine, Salt Lake City (Goldgar); Department of Oncological Sciences, Huntsman Cancer Institute, University of Utah School of Medicine, Salt Lake City (Buys); Vilnius University Hospital Santariskiu Clinics, Hematology, Oncology, and Transfusion Medicine Center, Department of Molecular and Regenerative Medicine, State Research Institute Innovative Medicine Center, Vilnius, Lithuania (Janavičius); Latvian Biomedical Research and Study Centre, Riga, Latvia (Tihomirova); Department of Medical Oncology, Beth Israel Deaconess Medical Center, Boston, Massachusetts (Tung); Department of Genetics, University of Pretoria, Pretoria, South Africa (Dorfling, van Rensburg); Department of Population Sciences, Beckman Research Institute of City of Hope, Duarte, California (Steele, Neuhausen, Ding);

Departments of Oncology or Clinical Genetics, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark (Ejlertsen, Gerdes); Center for Genomic Medicine, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark (Hansen); Oncology Service, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain (Ramón y Cajal); Human Genetics Group, Spanish National Cancer Centre (CNIO), and Biomedical Network on Rare Diseases (CIBERER), Madrid, Spain (Osorio); Human Genetics Group and Genotyping Unit, Spanish National Cancer Centre (CNIO), and Biomedical Network on Rare Diseases (CIBERER), Madrid, Spain (Benitez); Hospital clinico Universitario "Lozano Blesa," Instituto de investigación sanitaria de Aragón (IIS), Zaragoza, Spain (Godino); Molecular Genetics Laboratory (Department of Genetics), Cruces University Hospital Barakaldo, Bizkaia, Spain (Tejada); Institute of Biology and Molecular Genetics. Universidad de Valladolid (IBGM-UVA), Valladolid, Spain (Duran); Clinical Cancer Genetics, City of Hope Clinical Cancer Genetics Community Research Network, Duarte, California (Weitzel, Bobolis, Sand, Fontaine); Unit of Genetic Counselling, Medical Oncology Department, Istituto Nazionale Tumori Regina Elena, Rome, Italy (Savarese); Department of Medical Science, University of Turin, and AO Città della Salute e della Scienza, Turin, Italy (Pasini); Unit of Medical Genetics, Department of Preventive and Predictive Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori (INT), Milan, Italy (Peissel, Zaffaroni, Scuvera, Manoukian); Division of Cancer Prevention and Genetics, Istituto Europeo di Oncologia, Milan, Italy (Bonanni); AO Città della Salute e della Scienza, Turin, Italy (Vignolo-Lutati); Department of Molecular Medicine, University La Sapienza, Rome, Italy (Giannini); Department of Experimental Oncology, Istituto Europeo di Oncologia, Milan, Italy (Bernard); Cogentech Cancer Genetic Test Laboratory, Milan, Italy (Bernard, Pensotti); Institute of Medical Genetics, Catholic University, "A. Gemelli" Hospital, Rome, Italy (Genuardi); Unit of Molecular Bases of Genetic Risk and Genetic Testing, Department of Preventive and Predictive Medicine, Fondazione IRCCS Istituto Nazionale Tumori (INT), Milan, Italy (Radice); IFOM, Fondazione Istituto FIRC di Oncologia Molecolare, Milan, Italy (Radice, Pensotti); Cancer Bioimmunotherapy Unit, Centro di Riferimento Oncologico, IRCCSCRO Aviano National Cancer Institute, Aviano (PN), Italy (Dolcetti); Unit of Hereditary Cancer, IRCCS AOU San Martino–IST Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy (Gismondi); Molecular Diagnostics Laboratory, IRRP, National Centre for Scientific Research "Demokritos" Aghia Paraskevi Attikis, Athens, Greece (Yannoukakos, Fostira); Instituto de Genética Humana, Pontificia Universidad Javeriana, Bogotá, Colombia (Torres); Molecular Genetics of Breast Cancer, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany (Torres, Rashid, Hamann); Department of Basic Sciences, Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCH & RC), Lahore, Pakistan (Rashid); Genetic Medicine, Manchester Academic Health Sciences Centre, Central Manchester University Hospitals, NHS Foundation Trust, Manchester, United Kingdom (Evans); Oncogenetics Team, Institute of Cancer Research and Royal Marsden, NHS Foundation Trust, London, United Kingdom (Eeles); Ferguson-Smith Centre for Clinical Genetics, Yorkhill Hospitals, Glasgow, United Kingdom (Davidson); Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton, United Kingdom (Eccles); West Midlands Regional Genetics Service, Birmingham Women's Hospital Healthcare NHS Trust, Edgbaston, Birmingham, United Kingdom (Cole); Sheffield Clinical Genetics Service, Sheffield Children's Hospital,

Sheffield, United Kingdom (Cook); Department of Clinical Genetics, Royal Devon and Exeter Hospital, Exeter, United Kingdom (Brewer); Clinical Genetics Department, St Georges Hospital, University of London, United Kingdom (Hodgson); Northern Ireland Regional Genetics Centre, Belfast City Hospital, Belfast, United Kingdom (Morrison); Oxford Regional Genetics Service, Churchill Hospital, Oxford, United Kingdom (Walker); South East of Scotland Regional Genetics Service, Western General Hospital, Edinburgh, United Kingdom (Porteous); Academic Unit of Clinical and Molecular Oncology, Trinity College Dublin and St James's Hospital, Dublin, Eire (Kennedy); South East Thames Regional Genetics Service, Guy's Hospital London, United Kingdom (Izatt); Yorkshire Regional Genetics Service, Leeds, United Kingdom (Adlard); South West Regional Genetics Service, Bristol, United Kingdom (Donaldson); Department of Hematology and Oncology, University of Kansas Medical Center, Kansas City (Sharma); Center for Hereditary Breast and Ovarian Cancer, Center for Integrated Oncology (CIO), and Center for Molecular Medicine Cologne (CMMC), Medical Faculty, University of Cologne and University Hospital Cologne, Cologne, Germany (Schmutzler, Wappenschmidt, Becker, Rhiem, Hahnen); Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, Germany (Engel); Department of Gynaecology and Obstetrics, Division of Tumor Genetics, Klinikum rechts der Isar, Technical University Munich, Munich, Germany (Meindl, Engert, Ditsch); Department of Gynecology and Obstetrics, University Medical Center Schleswig-Holstein, Campus Kiel, Germany (Arnold, Mundhenke); Institute of Human Genetics, University Medical Center Schleswig-Holstein, Campus Kiel, Germany (Plendl); Department of Gynaecology and Obstetrics, University Hospital Düsseldorf, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany (Niederacher, Fleisch); Institute of Human Genetics, Department of Human Genetics, University Hospital Heidelberg, Heidelberg, Germany (Sutter, Bartram, Dikow); Department of Gynaecology and Obstetrics, University Hospital Ulm, Ulm, Germany (Wang-Gohrke); Institute of Cell and Molecular Pathology, Hannover Medical School, Hannover, Germany (Gadzicki, Steinemann); Department of Gynaecology and Obstetrics, University Hospital Carl Gustav Carus, Technical University Dresden, Dresden, Germany (Kast); Institute of Human Genetics, Technical University Dresden, Dresden, Germany (Beer); Institute of Human Genetics, Campus Virchov Klinikum, Charite Berlin, Berlin, Germany (Varon-Mateeva); Centre of Familial Breast and Ovarian Cancer, Department of Medical Genetics, Institute of Human Genetics, University of Würzburg, Würzburg, Germany (Gehrig); Institute of Human Genetics, University of Regensburg, Regensburg, Germany (Weber); Institut Curie, Department of Tumour Biology, Paris, France (Stoppa-Lyonnet, Houdayer, Belotti, Gauthier-Villars); Institut Curie, INSERM U830, Paris, France (Stoppa-Lyonnet); Université Paris Descartes, Sorbonne Paris Cité, France (Stoppa-Lyonnet, Houdayer); Unité Mixte de Génétique Constitutionnelle des Cancers Fréquents, Hospices Civils de Lyon–Centre Léon Bérard, Lyon, France (Sinilnikova, Boutry-Kryza); INSERM U1052, CNRS UMR5286, Université Lyon 1, Centre de Recherche en Cancérologie de Lyon, Lyon, France (Sinilnikova, Mazoyer, Damiola); Université Lyon 1, CNRS UMR5558, Lyon, France (Lasset); Unité de Prévention et d'Epidémiologie Génétique, Centre Léon Bérard, Lyon, France (Lasset); Département Oncologie Génétique, Prévention et Dépistage, INSERM CIC-P9502, Institut Paoli-Calmettes/Université d'Aix-Marseille II, Marseille, France (Sobol); Laboratoire d'Oncologie Moléculaire Humaine, Centre Oscar Lambret, Lille,

France (Peyrat); Unité d'Oncogénétique, CLCC Paul Strauss, Strasbourg, France (Muller, Fricker); Service de Génétique Biologique-Histologie-Biologie du Développement et de la Reproduction, Centre Hospitalier Universitaire de Besançon, Besançon, France (Collonge-Rame); Service de Génétique, Centre Hospitalier Universitaire Bretonneau, Tours, France (Mortemousque); Oncogénétique Clinique, Hôpital René Huguenin/Institut Curie, Saint-Cloud, France (Nogues); Laboratoire d'Oncogénétique, Hôpital René Huguenin/Institut Curie, Saint-Cloud, France (Rouleau); Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC (Isaacs); Center for Medical Genetics, Ghent University, Ghent, Belgium (De Paepe, Poppe, Claes, De Leeneer); GOG Statistical and Data Center, Buffalo, New York (Piedmonte); Evanston Hospital, Evanston, Illinois (Rodriguez); Tufts University, Medford, Massachusetts (Wakely); University of North Carolina, Chapel Hill (Boggess); New York University, New York, New York (Blank); Ohio State, Good Samaritan Hospital, Cincinnati (Basil); Yale University School of Medicine, New Haven, Connecticut (Azodi); Molecular Oncology Laboratory, Hospital Clinico San Carlos, IdISSC, Madrid, Spain (Caldes, de la Hoya, Romero); Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland (Nevanlinna); Department of Clinical Genetics, Helsinki University Central Hospital, Helsinki, Finland (Aittomäki); Department of Genetics, University Medical Center, Groningen University, Groningen, The Netherlands (van der Hout, Oosterwijk); Family Cancer Clinic, Netherlands Cancer Institute, Amsterdam, The Netherlands (Hogervorst, Verhoef); Department of Clinical Genetics, Family Cancer Clinic, Erasmus University Medical Center, Rotterdam, The Netherlands (Collée); Department of Medical Oncology, Family Cancer Clinic, Erasmus University Medical Center, Rotterdam, The Netherlands (Seynaeve); Department of Clinical Genetics, VU University Medical Centre, Amsterdam, The Netherlands (Gille); Department of Human Genetics and Department of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands (Wijnen); Department of Clinical Genetics and GROW, School for Oncology and Developmental Biology, MUMC, Maastricht, The Netherlands (Garcia); Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands (Kets, Mensenkamp); Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands (Ausems); Department of Clinical Genetics, Academic Medical Center, Amsterdam, The Netherlands (Aalfs); Department of Human Genetics and Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands (Devilee); Hong Kong Hereditary Breast Cancer Family Registry, Hong Kong (Kwong); Cancer Genetics Center, Hong Kong Sanatorium and Hospital, Hong Kong (Kwong); Department of Surgery, University of Hong Kong, Hong Kong (Kwong); Department of Molecular Genetics, National Institute of Oncology, Budapest, Hungary (Olah, Papp); Oncogenetics Laboratory, Vall d'Hebron Institute of Oncology (VHIO), Universitat Autonoma de Barcelona, Barcelona, Spain (Diez); University Hospital of Vall d'Hebron, Barcelona, Spain (Diez); Molecular Diagnostic Unit, Hereditary Cancer Program, IDIBELL-Catalan Institute of Oncology, Barcelona, Spain (Lazaro); Genetic Counseling Unit, Hereditary Cancer Program, IDIBGI-Catalan Institute of Oncology, Girona, Spain (Darder); Genetic Counseling Unit, Hereditary Cancer Program, IDIBELL-Catalan Institute of Oncology, Barcelona, Spain (Blanco, Salinas); Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland (Jakubowska, Lubinski, Gronwald,

Jaworska-Bieniek, Durda, Sukiennicki, Huzarski, Byrski, Cybulski, Toloczko-Grabarek, Zlowocka-Perlowska); Postgraduate School of Molecular Medicine, Warsaw Medical University, Warsaw, Poland (Jaworska-Bieniek); Department of Surgical Gynecology and Gynecological Oncology of Adults and Adolescents, Pomeranian Medical University, Szczecin, Poland (Menkiszak); Department of Pathology, Landspitali University Hospital, Reykjavík, Iceland (Arason, Barkardottir); BMC, Faculty of Medicine, University of Iceland, Reykjavik, Iceland (Arason, Barkardottir); Canada Research Chair in Oncogenetics, Cancer Genomics Laboratory, Centre Hospitalier Universitaire de Québec Research Center, Quebec City, Quebec, Canada (Simard); Laval University, Quebec City, Quebec, Canada (Simard); Medical Genetics Division, Centre Hospitalier Universitaire de Québec, Quebec City, Quebec, Canada (Laframboise); Immunology and Molecular Oncology Unit, Veneto Institute of Oncology, IOV-IRCCS, Padua, Italy (Laframboise, Montagna, Agata, Alducci); Department of Genetics, Portuguese Oncology Institute, Porto, Portugal (Peixoto, Teixeira); Biomedical Sciences Institute (ICBAS), University of Porto, Portugal (Teixeira); Department of Surgery, Soonchunhyang University and Hospital, Seoul, Korea (Lee); Department of Preventive Medicine, Seoul National University College of Medicine and Cancer Research Institute, Seoul National University, Seoul, Korea (Park); Department of Surgery, Daerim St Mary's Hospital, Seoul, Korea (Kim); Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota (Couch, Guidugli, Wang); Department of Health Sciences Research, Mayo Clinic, Rochester, Minnesota (Couch, Lindor, Pankratz); Program in Cancer Genetics, Departments of Human Genetics and Oncology, McGill University, Montreal, Quebec, Canada (Tischkowitz); Department of Medical Genetics, University of Cambridge, Cambridge, United Kingdom (Tischkowitz); Department of Cancer Epidemiology and Genetics, Masaryk Memorial Cancer Institute and MF MU, Brno, Czech Republic (Foretova); Clinical Genetics Service, Cancer Biology and Genetics Program, Memorial Sloan-Kettering Cancer Center, New York, New York (Vijai, Offit); Clinical Genetics Service, Memorial Sloan-Kettering Cancer Center, New York, New York (Robson, Rau-Murthy, Kauff); Department of OB/GYN and Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria (Fink-Retter, Singer, Rappaport, Gschwantler-Kaulich, Pfeiler, Tea, A. Berger); Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, Maryland (Greene, Mai); N. N. Petrov Institute of Oncology, St Petersburg, Russia (Imyanitov); Divison of Human Cancer Genetics, Departments of Internal Medicine and Molecular Virology, Immunology and Medical Genetics, Comprehensive Cancer Center, Ohio State University, Columbus (Toland); Divison of Human Genetics, Department of Internal Medicine, Comprehensive Cancer Center, Ohio State University, Columbus (Senter); Department of Clinical Genetics, Vejle Hospital, Vejle, Denmark (Bojesen, Skytte); Section of Molecular Diagnostics, Department of Clinical Biochemistry, Aalborg University Hospital, Aalborg, Denmark (Pedersen); Department of Clinical Genetics, Aarhus University Hospital, Aarhus N, Denmark (Sunde, Jensen); Department of Clinical Genetics, Odense University Hospital, Odense C, Denmark (Thomassen, Moeller, Kruse); Section of Genetic Oncology, Department of Oncology, University of Pisa and University Hospital of Pisa, Pisa, Italy (Caligo, Aretini); Cancer Research Initiatives Foundation, Sime Darby Medical Centre, Subang Jaya, Malaysia (Teo); Department of Surgery, Faculty of Medicine, University Malaya Cancer Research Institute, University Malaya, Kuala Lumpur,

Malaysia (Teo); NorthShore University HealthSystem, Department of Medicine, Evanston, Illinois (Selkirk, Hulick); Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital and University of Toronto, Toronto, Ontario, Canada (Andrulis).

Additional Contributions

We thank Jinbo Chen, PhD, of the University of Pennsylvania for her valuable input regarding appropriate statistical methods. Dr Chen did not receive any financial compensation for her role in this work. We dedicate this article to the memory of Dr Olga M. Sinilnikova, who contributed substantively to this work by contributing her knowledge of the mutations and domains studied herein, as well as advancing our understanding of nonsense-mediated decay of *BRCA1* and *BRCA2*.

Author Contributions

Dr Rebbeck had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Wan, Mitra, Nathanson, and Rebbeck conducted and are responsible for the data analysis.

Study concept and design: Rebbeck, Chenevix-Trench, Easton, Antoniou, Nathanson, Zidan, Goldgar, Evans, Varon-Mateeva, Weber, Paepe, Lee, Park, Gschwantler-Kaulich.

Acquisition, analysis, or interpretation of data: Rebbeck, Mitra, Wan, Sinilnikova, Healey, McGuffog, Mazoyer, Chenevix-Trench, Easton, Nathanson, Laitman, Kushnir, Paluch-Shimon, R. Berger, Zidan, Friedman, Ehrencrona, Stenmark-Askmalm, Einbeigi, Loman, Harbst, Rantala, Melin, Huo, Olopade, Seldon, Ganz, Nussbaum, Chan, Odunsi, Gayther, Domchek, Arun, Lu, Mitchell, Karlan, Walsh, Lester, Godwin, Pathak, Ross, Daly, Whittemore, John, Miron, Terry, Chung, Goldgar, Buys, Janavicius, Tihomirova, Tung, Dorfling, van Rensburg, Steele, Neuhausen, Ding, Ejlertsen, Gerdes, Hansen, Cajal, Osorio, Benitez, Godino, Tejada, Duran, Weitzel, Bobolis, Sand, Fontaine, Savarese, Pasini, Peissel, Bonanni, Zaffaroni, Vignolo-Lutati, Scuvera, Giannini, Bernard, Genuardi, Radice, Dolcetti, Manoukian, Pensotti, Gismondi, Yannoukakos, Fostira, Garber, Torres, Rashid, Hamann, Peock, Frost, Platte, Evans, Eeles, Davidson, Eccles, Cole, Cook, Brewer, Hodgson, Morrison, Walker, Porteous, Kennedy, Izatt, Adlard, Donaldson, Ellis, Sharma, Schmutzler, Wappenschmidt, Becker, Rhiem, Hahnen, Engel, Meindl, Engert, Ditsch, Arnold, Plendl, Mundhenke, Niederacher, Fleisch, Sutter, Bartram, Dikow, Wang-Gohrke, Gadzicki, Steinemann, Kast, Beer, Gehrig, Stoppa-Lyonnet, Houdayer, Belotti, Gauthier-Villars, Damiola, Boutry-Kryza, Lasset, Sobol, Peyrat, Muller, Fricker, Collonge-Rame, Mortemousque, Nogues, Rouleau, Isaacs, Poppe, Claes, De Leeneer, Piedmonte, Rodriguez, Wakely, Boggess, Blank, Basil, Azodi, Phillips, Caldes, de la Hoya, Romero, Nevanlinna, Aittomaki, van der Hout, Hogervorst, Verhoef, Collee, Seynaeve, Oosterwijk, Gille, Wijnen, Garcia, Kets, Ausems, Aalfs, Devilee, Mensenkamp, Kwong, Olah, Papp, Diez, Lazaro, Darder, Blanco, Salinas, Jakubowska, Lubinski, Gronwald, Jaworska-Bieniek, Durda, Sukiennicki, Huzarski, Byrski, Cybulski, Toloczko-Grabarek, Zlowocka-Perlowska, Menkiszak, Arason, Barkardottir, Simard, Laframboise, Montagna, Agata, Alducci, Peixoto, Teixeira, Spurdle, Park, Kim, Friebel, Couch, Lindor, Pankratz, Guidugli, Wang,

Tischkowitz, Foretova, Vijai, Offit, Robson, Rau-Murthy, Kauff, Fink-Retter, Singer, Rappaport, Pfeiler, Tea, A. Berger, Greene, Mai, Imyanitov, Toland, Senter, Bojesen, Pedersen, Skytte, Sunde, Thomassen, Møller, Kruse, Jensen, Caligo, Aretini, Teo, Selkirk, Hulick, Andrulis.

Drafting of the manuscript: Rebbeck, Mitra, Chenevix-Trench, Nathanson, Zidan, Chan, Tejada, Pasini, Garber, Rashid, Evans, Gehrig, Belotti, Collonge-Rame, Nogues, Paepe, Blank, Kets, Lubinski, Jaworska-Bieniek, Durda, Sukiennicki, Huzarski, Toloczko-Grabarek, Lee, Kim, Rau-Murthy.

Critical revision of the manuscript for important intellectual content: Rebbeck, Mitra, Wan, Sinilnikova, Healey, McGuffog, Mazoyer, Chenevix-Trench, Easton, Antoniou, Nathanson, Laitman, Kushnir, Paluch-Shimon, R. Berger, Zidan, Friedman, Ehrencrona, Stenmark-Askmalm, Einbeigi, Loman, Harbst, Rantala, Melin, Huo, Olopade, Seldon, Ganz, Nussbaum, Odunsi, Gayther, Domchek, Arun, Lu, Mitchell, Karlan, Walsh, Lester, Godwin, Pathak, Ross, Daly, Whittemore, John, Miron, Terry, Chung, Goldgar, Buys, Janavicius, Tihomirova, Tung, Dorfling, van Rensburg, Steele, Neuhausen, Ding, Ejlertsen, Gerdes, Hansen, Cajal, Osorio, Benitez, Godino, Duran, Weitzel, Bobolis, Sand, Fontaine, Savarese, Peissel, Bonanni, Zaffaroni, Vignolo-Lutati, Scuvera, Bernard, Genuardi, Radice, Dolcetti, Manoukian, Pensotti, Gismondi, Yannoukakos, Fostira, Garber, Torres, Hamann, Peock, Frost, Platte, Evans, Eeles, Davidson, Eccles, Cole, Cook, Brewer, Hodgson, Morrison, Walker, Porteous, Kennedy, Izatt, Adlard, Donaldson, Ellis, Sharma, Schmutzler, Wappenschmidt, Becker, Rhiem, Hahnen, Engel, Meindl, Engert, Ditsch, Arnold, Plendl, Mundhenke, Niederacher, Fleisch, Sutter, Bartram, Dikow, Wang-Gohrke, Gadzicki, Steinemann, Kast, Beer, Varon-Mateeva, Weber, Stoppa-Lyonnet, Houdayer, Gauthier-Villars, Damiola, Boutry-Kryza, Lasset, Sobol, Peyrat, Muller, Fricker, Mortemousque, Rouleau, Isaacs, Poppe, Claes, De Leeneer, Piedmonte, Rodriguez, Wakely, Boggess, Blank, Basil, Azodi, Phillips, Caldes, de la Hoya, Romero, Nevanlinna, Aittomaki, van der Hout, Hogervorst, Verhoef, Collee, Seynaeve, Oosterwijk, Gille, Wijnen, Garcia, Ausems, Aalfs, Devilee, Mensenkamp, Kwong, Olah, Papp, Diez, Lazaro, Darder, Blanco, Salinas, Jakubowska, Gronwald, Byrski, Cybulski, Zlowocka-Perlowska, Menkiszak, Arason, Barkardottir, Simard, Laframboise, Montagna, Agata, Alducci, Peixoto, Teixeira, Spurdle, Park, Friebel, Couch, Lindor, Pankratz, Guidugli, Wang, Tischkowitz, Foretova, Vijai, Offit, Robson, Kauff, Fink-Retter, Singer, Rappaport, Gschwantler-Kaulich, Pfeiler, Tea, A. Berger, Greene, Mai, Imyanitov, Toland, Senter, Bojesen, Pedersen, Skytte, Sunde, Thomassen, Møller, Kruse, Jensen, Caligo, Aretini, Teo, Selkirk, Hulick, Andrulis.

Statistical analysis: Rebbeck, Mitra, Wan, Easton, Antoniou, Nathanson, Zidan, Whittemore, Goldgar, Kennedy, Park, Pankratz.

Obtained funding: Rebbeck, Chenevix-Trench, Easton, Antoniou, Nathanson, Domchek, Ross, Daly, John, Buys, van Rensburg, Benitez, Genuardi, Yannoukakos, Schmutzler, Weber, Mortemousque, Azodi, Phillips, de la Hoya, Nevanlinna, Kwong, Olah, Salinas, Simard, Kim, Lindor, Offit, Kruse, Caligo.

Administrative, technical, or material support: Rebbeck, Healey, McGuffog, Nathanson, Laitman, Kushnir, Paluch-Shimon, Zidan, Stenmark-Askmalm, Einbeigi, Harbst, Melin, Huo, Seldon, Ganz, Odunsi, Gayther, Domchek, Arun, Lu, Karlan, Lester, Godwin, Miron, Dorfling, van Rensburg, Steele, Ejlertsen, Hansen, Cajal, Osorio, Duran, Weitzel, Sand, Bernard, Pensotti, Gismondi, Yannoukakos, Fostira, Garber, Rashid, Peock, Frost, Platte, Evans, Eeles, Eccles, Cole, Brewer, Morrison, Donaldson, Ellis, Sharma, Schmutzler, Wappenschmidt, Becker, Rhiem, Hahnen, Meindl, Engert, Ditsch, Arnold, Mundhenke, Niederacher, Fleisch, Sutter, Bartram, Dikow, Wang-Gohrke, Steinemann, Kast, Gehrig, Weber, Houdayer, Belotti, Damiola, Peyrat, Isaacs, Claes, De Leeneer, Piedmonte, Azodi, de la Hoya, Romero, Nevanlinna, Aittomaki, van der Hout, Hogervorst, Collee, Garcia, Devilee, Mensenkamp, Kwong, Olah, Papp, Lazaro, Salinas, Jakubowska, Lubinski, Huzarski, Byrski, Cybulski, Toloczko-Grabarek, Zlowocka-Perlowska, Menkiszak, Arason, Barkardottir, Simard, Laframboise, Montagna, Agata, Alducci, Peixoto, Teixeira, Kim, Friebel, Couch, Guidugli, Wang, Foretova, Vijai, Offit, Rau-Murthy, Rappaport, Gschwantler-Kaulich, Pfeiler, A. Berger, Greene, Mai, Toland, Senter, Bojesen, Pedersen, Møller, Kruse, Jensen, Teo, Hulick, Andrulis.

Study supervision: Rebbeck, Mitra, Antoniou, Nathanson, Zidan, Friedman, John, van Rensburg, Rashid, Sobol, Paepe, Caldes, Gille, Lazaro, Laframboise, Agata, Lee, Singer, Kruse.

Conflict of Interest Disclosures

All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Mitra reported having received grants from the National Institutes of Health (NIH). Ms McGuffog reported having receiving salary funded by a grant from Cancer Research UK. Dr Easton reported having received grants from Cancer Research UK. Dr Harbst reported having received grants from the Swedish Cancer Society. Dr Nussbaum reported having received grants from NIH and US Department of Defense and other support from Complete Genomics, Personalis, Oblon Spivak Law Firm, Guidepont Global, Monness, and El Camino Hospital. Dr Ross reported having received grants from the National Cancer Institute (NCI). Dr Daly reported having received grants from NCI. Dr John reported having received grants from the Cancer Prevention Institute of California. Dr Goldgar reported having a patent for *BRCA1* and *BRCA2* with royalties paid. Dr Dorfling reported having received grants from the Cancer Association of South Africa (CANSA). Dr van Rensburg reported having received grants from CANSA. Ms Steele reported having received grants and support from NIH. Dr Ejlertsen reported having received grants from Amgen, Novartis, and Roche. Dr Radice reported having received grants from the Italian Association for Cancer Research. Dr Manoukian reported having received grants from the Fondazione IRCCS Istituto Nazionale Tumori. Dr Garber reported having received research support from Myriad Genetics Laboratories. Dr Eeles reported having received travel and education support from Succinct Health Communications and Janssen Pharmaceuticals. Dr Davidson reported having received funding from from Cancer Research UK. Dr Cook reported having been an EMBRACE study coordinator. Dr Schmutzler reported having served on a board and speakers' bureau for and having received travel expenses from AstraZeneca and having a patent for RMD51C with royalties paid. Dr Engel reported having

received grants from German Cancer Aid. Dr Devilee reported having patents for a diagnostic kit for determining a predisposition for breast and ovarian cancer. Dr Simard reported having been named as inventor on *BRCA1/2* gene patents but not receiving royalties. Dr Couch reported being co-holder of a patent on the *BRCA2* gene with royalties paid. Dr Robson reported having received grants from the Breast Cancer Research Foundation. Dr Toland reported having received grants from NIH and the American Cancer Society and travel support from NIH and serving on a board for NIH. No other disclosures were reported.

References

1. Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. Science. 1994; 266(5182):66–71. [PubMed: 7545954]
2. Wooster R, Neuhausen SL, Mangion J, et al. Localization of a breast cancer susceptibility gene, *BRCA2*, to chromosome 13q12-13. Science. 1994; 265 (5181):2088–2090. [PubMed: 8091231]
3. Gayther SA, Warren W, Mazoyer S, et al. Germline mutations of the *BRCA1* gene in breast and ovarian cancer families provide evidence for a genotype-phenotype correlation. Nat Genet. 1995; 11(4):428–433. [PubMed: 7493024]
4. Thompson D, Easton D. Breast Cancer Linkage Consortium. Variation in *BRCA1* cancer risks by mutation position. Cancer Epidemiol Biomarkers Prev. 2002; 11(4):329–336. [PubMed: 11927492]
5. Gayther SA, Mangion J, Russell P, et al. Variation of risks of breast and ovarian cancer associated with different germline mutations of the *BRCA2* gene. Nat Genet. 1997; 15(1):103–105. [PubMed: 8988179]
6. Perrin-Vidoz L, Sinilnikova OM, Stoppa-Lyonnet D, Lenoir GM, Mazoyer S. The nonsense-mediated mRNA decay pathway triggers degradation of most *BRCA1* mRNAs bearing premature termination codons. Hum Mol Genet. 2002; 11(23):2805–2814. [PubMed: 12393792]
7. Ware MD, DeSilva D, Sinilnikova OM, Stoppa-Lyonnet D, Tavtigian SV, Mazoyer S. Does nonsense-mediated mRNA decay explain the ovarian cancer cluster region of the *BRCA2* gene? Oncogene. 2006; 25(2):323–328. [PubMed: 16170354]
8. Dine J, Deng CX. Mouse models of *BRCA1* and their application to breast cancer research. Cancer Metastasis Rev. 2013; 32(1–2):25–37. [PubMed: 23093327]
9. Evers B, Jonkers J. Mouse models of *BRCA1* and *BRCA2* deficiency: past lessons, current understanding and future prospects. Oncogene. 2006; 25(43):5885–5897. [PubMed: 16998503]
10. Chenevix-Trench G, Milne RL, Antoniou AC, Couch FJ, Easton DF, Goldgar DE. CIMBA. An international initiative to identify genetic modifiers of cancer risk in *BRCA1* and *BRCA2* mutation carriers: the Consortium of Investigators of Modifiers of *BRCA1* and *BRCA2* (CIMBA). Breast Cancer Res. 2007; 9(2):104. [PubMed: 17466083]
11. Claes K, Poppe B, Machackova E, et al. Differentiating pathogenic mutations from polymorphic alterations in the splice sites of *BRCA1* and *BRCA2*. Genes Chromosomes Cancer. 2003; 37 (3): 314–320. [PubMed: 12759930]
12. Goldgar DE, Easton DF, Deffenbaugh AM, Monteiro AN, Tavtigian SV, Couch FJ. Breast Cancer Information Core (BIC) Steering Committee. Integrated evaluation of DNA sequence variants of unknown clinical significance: application to *BRCA1* and *BRCA2*. Am J Hum Genet. 2004; 75(4): 535–544. [PubMed: 15290653]
13. Chenevix-Trench G, Healey S, Lakhani S, et al. kConFab Investigators. . Genetic and histopathologic evaluation of *BRCA1* and *BRCA2* DNA sequence variants of unknown clinical significance. Cancer Res. 2006; 66(4):2019–2027. [PubMed: 16489001]
14. Brzovic PS, Rajagopal P, Hoyt DW, King MC, Klevit RE. Structure of a BRCA1-BARD1 heterodimeric RING-RING complex. Nat Struct Biol. 2001; 8(10):833–837. [PubMed: 11573085]
15. Bienstock RJ, Darden T, Wiseman R, Pedersen L, Barrett JC. Molecular modeling of the amino-terminal zinc ring domain of *BRCA1*. Cancer Res. 1996; 56(11):2539–2545. [PubMed: 8653694]

16. Huyton T, Bates PA, Zhang X, Sternberg MJ, Freemont PS. The *BRCA1* C-terminal domain: structure and function. Mutat Res. 2000; 460(3–4):319–332. [PubMed: 10946236]
17. Williams RS, Green R, Glover JN. Crystal structure of the BRCT repeat region from the breast cancer-associated protein BRCA1. Nat Struct Biol. 2001; 8(10):838–842. [PubMed: 11573086]
18. Bork P, Hofmann K, Bucher P, Neuwald AF, Altschul SF, Koonin EV. A superfamily of conserved domains in DNA damage-responsive cell cycle checkpoint proteins. FASEB J. 1997; 11(1):68–76. [PubMed: 9034168]
19. Wu LC, Wang ZW, Tsan JT, et al. Identification of a RING protein that can interact in vivo with the *BRCA1* gene product. Nat Genet. 1996; 14(4):430–440. [PubMed: 8944023]
20. Palacios IM. Nonsense-mediated mRNA decay: from mechanistic insights to impacts on human health. Brief Funct Genomics. 2013; 12(1):25–36. [PubMed: 23148322]
21. Buisson M, Anczuków O, Zetoune AB, Ware MD, Mazoyer S. The 185delAG mutation (c. 68_69delAG) in the *BRCA1* gene triggers translation reinitiation at a downstream AUG codon. Hum Mutat. 2006; 27 (10):1024–1029. [PubMed: 16941470]
22. [Accessed March 9, 2015] Pfam 27.0 database. http://pfam.xfam.org
23. Lin DY, Wei LJ. The robust inference for the Cox proportional hazards model. J Am Stat Assoc. 1989; 84:1074–1078.
24. Therneau, T.; Grambsch, P. Modeling Survival Data: Extending the Cox Model. New York, NY: Springer Verlag; 2000. p. 169-229.
25. Anczuków O, Ware MD, Buisson M, et al. Does the nonsense-mediated mRNA decay mechanism prevent the synthesis of truncated BRCA1, CHK2, and p53 proteins? Hum Mutat. 2008; 29(1):65–73. [PubMed: 17694537]
26. Antoniou AC, Cunningham AP, Peto J, et al. The BOADICEA model of genetic susceptibility to breast and ovarian cancers: updates and extensions. Br J Cancer. 2008; 98(8):1457–1466. [PubMed: 18349832]
27. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol. 1995; 57:289–300.
28. Bignell G, Micklem G, Stratton MR, Ashworth A, Wooster R. The BRC repeats are conserved in mammalian BRCA2 proteins. Hum Mol Genet. 1997; 6(1):53–58. [PubMed: 9002670]
29. Thompson D, Easton D. Breast Cancer Linkage Consortium. Variation in cancer risks, by mutation position, in *BRCA2* mutation carriers. Am J Hum Genet. 2001; 68(2):410–419. [PubMed: 11170890]
30. Lubinski J, Phelan CM, Ghadirian P, et al. Cancer variation associated with the position of the mutation in the *BRCA2* gene. Fam Cancer. 2004; 3 (1):1–10. [PubMed: 15131399]
31. Ludwig T, Fisher P, Ganesan S, Efstratiadis A. Tumorigenesis in mice carrying a truncating Brca1 mutation. Genes Dev. 2001; 15(10):1188–1193. [PubMed: 11358863]
32. Antoniou AC, Sinilnikova OM, Simard J, et al. Genetic Modifiers of Cancer Risk in *BRCA1/2* Mutation Carriers Study (GEMO); Epidemiological Study of *BRCA1* and *BRCA2* Mutation Carriers (EMBRACE); German Consortium for Hereditary Breast and Ovarian Cancer (GCHBOC); Kathleen Cuningham Consortium for Research into Familial Breast Cancer (kConFab); Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA). . RAD51 135G-->C modifies breast cancer risk among *BRCA2* mutation carriers: results from a combined analysis of 19 studies. Am J Hum Genet. 2007; 81(6):1186–1200. [PubMed: 17999359]
33. Yang H, Jeffrey PD, Miller J, et al. *BRCA2* function in DNA binding and recombination from a BRCA2-DSS1-ssDNA structure. Science. 2002; 297 (5588):1837–1848. [PubMed: 12228710]
34. Domchek SM, Friebel TM, Singer CF, et al. Association of risk-reducing surgery in *BRCA1* or *BRCA2* mutation carriers with cancer risk and mortality. JAMA. 2010; 304(9):967–975. [PubMed: 20810374]

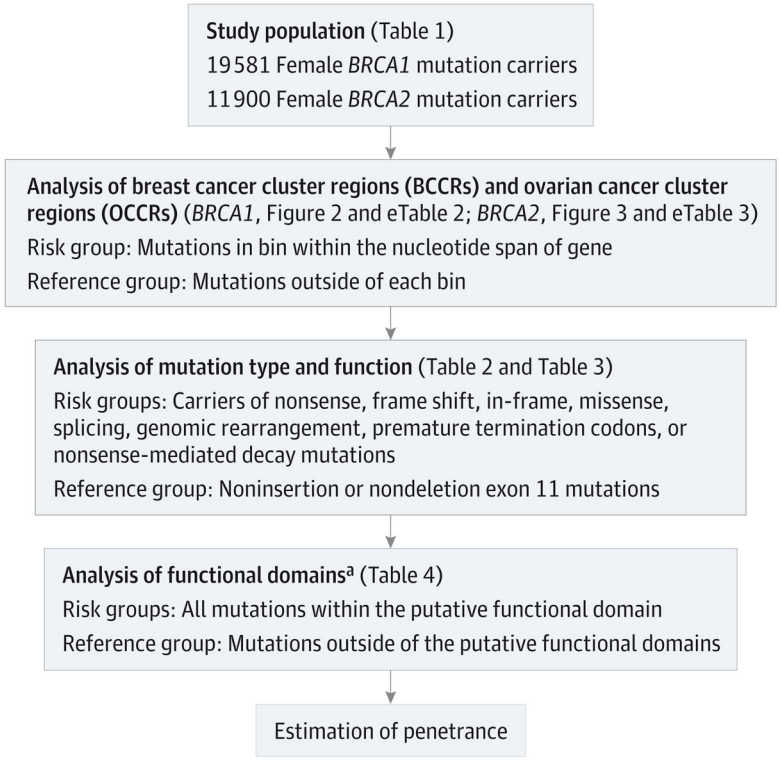

Figure 1. Analysis Workflow

Analyses undertaken are listed in the order in which they are presented in the text. [a]The functional domains were the RING, coiled coil, BRCT, BRC, DNA binding, oligonucleotide-binding folds, and tower domains.

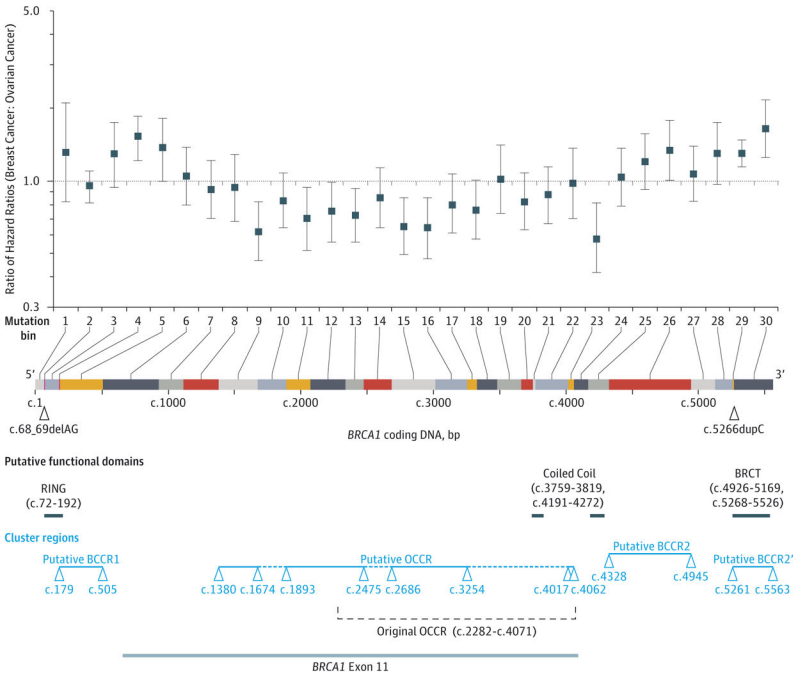

Figure 2. Hazard Ratio of Breast Cancer Relative to the Hazard Ratio of Ovarian Cancer by *BRCA1* Nucleotide Position

The graph shows the ratio of hazard ratios (blue data markers) and 95% CI (error bars) for the mutation bins defined across the span of the coding DNA sequence of the *BRCA1* gene. Black arrowheads under the bins indicate 2 founder mutations of clinical interest in the Ashkenazi Jewish population. Regions inferred to be breast cancer cluster regions (BCCRs) and ovarian cancer cluster regions (OCCRs) are shown at the bottom. Solid light blue lines indicate regions found to be statistically significant; dashed light blue lines indicate regions in the same direction of effect that were not statistically significant. eTable 2 in the Supplement lists the bins and risks used to define the BCCRs and OCCRs.

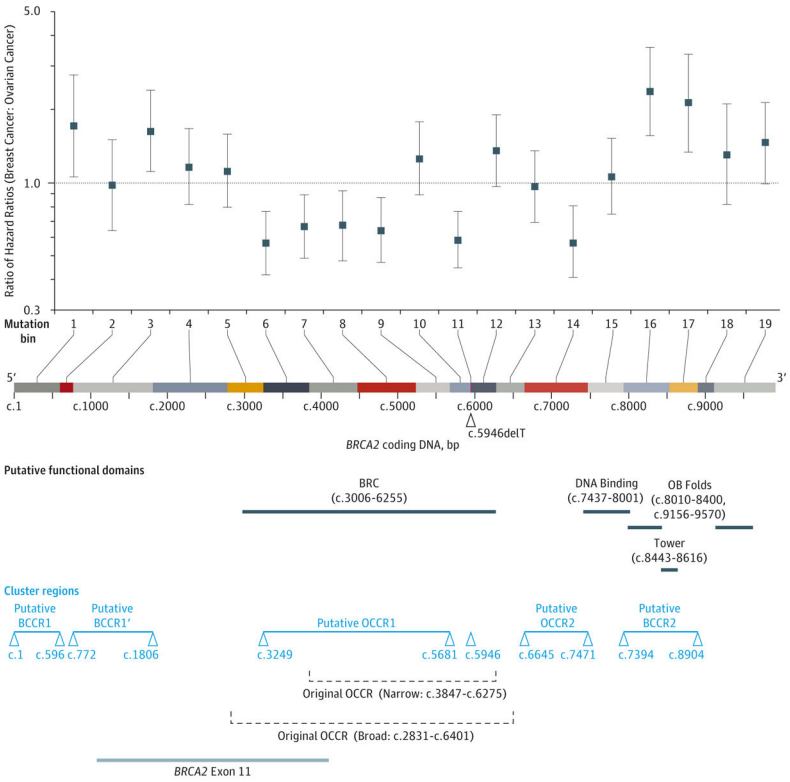

Figure 3. Hazard Ratio of Breast Cancer Relative to the Hazard Ratio of Ovarian Cancer by *BRCA2* Nucleotide Position

The graph shows the ratio of hazard ratios (blue data markers) and 95% CI (error bars) for the mutation bins defined across the span of the coding DNA sequence of the *BRCA2* gene. The black arrowhead under the bins indicates a founder mutation of clinical interest in the Ashkenazi Jewish population. The regions inferred to be breast cancer cluster regions (BCCRs) and ovarian cancer cluster regions (OCCRs) are shown at the bottom; the solid light blue lines indicate regions found to be statistically significant. eTable 3 in the Supplement lists the bins and risks used to define to define the BCCRs and OCCRs.

JAMA. Author manuscript; available in PMC 2015 August 15.

Table 1

Characteristics of Study Sample: Ascertainment, Diagnosis, Demographics, and Risk Factors

	***BRCA1* Mutation Carriers**			***BRCA2* Mutation Carriers**		
Variable	**No.**	**Median or Mean (Range)**	**SD**	**No.**	**Median or Mean (Range)**	**SD**
Women with breast cancer	10 093			6452		
Year of breast cancer diagnosis		1999 (1942–2011)			1999 (1937–2011)	
Mean age at breast cancer diagnosis, y		39.9 (17–85)	9.2		42.8 (17–86)	9.8
Women without breast cancer	9488			5448		
Mean age of women with no breast cancer diagnosis, y		41.0 (12–102)[a]	12.0		42.6 (13–94)[a]	13.1
Women with ovarian cancer	3358			954		
Year of ovarian cancer diagnosis		2001 (1949–2011)			2001 (1967–2010)	
Mean age at ovarian cancer diagnosis, y		50.0 (16–92)[b]	9.5		56.5 (19–89)[b]	9.9
Women without ovarian cancer	16 223			10 946		
Mean age of women with no ovarian cancer diagnosis, y		42.0 (12–102)[a]	12.0		45.4 (13–96)[a]	12.6
Race/ethnicity						
White	16 481			10 014		
African/African American	176			87		
Asian	392			404		
Hispanic	333			175		
Jewish	1800			971		
Other	399			249		
Parity, No. of live births		2.0 (0–14)	1.4		2.0 (0–14)	1.4

Variable	*BRCA1* Mutation Carriers			*BRCA2* Mutation Carriers		
	No.	Median or Mean (Range)	SD	No.	Median or Mean (Range)	SD
Age at menarche, y		13.0 (8–23)	1.5		13.0 (7–22)	1.6
Age at natural or surgical menopause, y		44.0 (16–68)	6.3		46.0 (14–68)	6.6

[a] Includes age at time of original family ascertainment for some women who were never diagnosed with cancer.

[b] Includes 3 germ cell carcinoma cases diagnosed before age 21 years.

Table 2

Mutation-Specific Risk Groups: Risks Relative to Noninsertion or Nondeletion Exon 11 Mutations

Group	Description	Mutation Types Included	NMD	Protein	No. (%) With Mutation	Breast Cancer, HR (95% CI)	No. of Women With Breast Cancer	Ovarian Cancer, HR (95% CI)	No. of Women With Ovarian Cancer
BRCA1 **(n = 19 581)**									
a	Exon 11 nonsense mutations	NS	Yes	No	1770 (9.0)	1 [Reference]	796	1 [Reference]	336
1	NMD	FS, NS, OF-GR, OF-SP	Yes	No	11 027 (56.3)	1.20 (1.08–1.33)	5469	0.94 (0.80–1.11)	2038
2	All premature termination mutations	FS, NS, OF-GR, OF-SP	Yes	No	14 453 (73.8)	1.25 (1.12–1.38)	7318	0.93 (0.79–1.09)	2524
3	Mutations before c.297 ATG presumed transcription reinitiation	FS, NS	No	No	2763 (14.1)	1.40 (1.12–1.74)	250	0.66 (0.46–0.95)	74
4	Not premature termination	MS, IF, GR, IF-SP, FS	No	Yes	5398 (27.6)	1.51 (1.34–1.70)	2986	0.73 (0.61–0.88)	781
5	All founder mutations	FS			5375 (27.5)	1.41 (1.23–1.61)	2698	0.72 (0.60–0.88)	850
5a	Founder mutation c.68_69delAG	FS		No	2324 (11.9)	1.14 (0.94–1.38)	1033	0.67 (0.51–0.87)	391
5b	Founder mutation c.5266dupC	FS		Yes	3051 (15.6)	1.63 (1.41–1.89)	1665	0.73 (0.57–0.92)	459
6	All missense mutations	MS		Yes	1620 (8.3)	1.40 (1.20–1.64)	899	0.73 (0.57–0.92)	241
6a	Missense mutations in RING domain (c. 72–192)	MS		Yes	1213 (6.2)	1.56 (1.32–1.84)	681	0.73 (0.56–0.96)	171
6b	Missense mutations in BRCT domain (c. 4866–5325)	MS		Yes	372 (1.9)	1.09 (0.82–1.45)	202	0.72 (0.48–1.09)	64
7	Missense mutations and in-frame deletions	MS+IF, IF-SP, IF-GR		Yes	1658 (8.5)	1.42 (1.22–1.66)	925	0.71 (0.56–0.91)	249
8	In-frame deletions (splice, single codon, large deletion)	IF, IF-SP, IF-GR		Yes	38 (0.2)	2.41 (1.41–4.11)	26	0.51 (0.15–1.76)	8
9	All premature termination codons not leading to NMD	FS, NS, OF-SP, OF-GR	No	Yes	3663 (18.7)	1.58 (1.38–1.80)	2000	0.74 (0.60–0.91)	520
BRCA2 **(n = 11 900)**									
a	Exon 11 nonsense mutations	NS	Yes	No	1001 (8.4)	1 [Reference]	534	1 [Reference]	155
1	NMD	FS, NS, OF-GR, OF-SP	Yes	No	9961 (83.7)	1.10 (0.95–1.27)	5383	0.78 (0.56–1.08)	803
2	Not premature termination codon	IF-S, IF-FS, NS	No	Yes	203 (1.7)	1.35 (0.92–1.97)	118	0.34 (0.13–0.88)	12
3	In-frame deletions (splice, frame shift)	IF-FS, IF-S	No	Yes	117 (1.0)	1.41 (0.85–2.35)	76	0.26 (0.08–0.88)	9
4	Premature termination codons in last exon not leading to NMD	FS, NS	No	Yes	86 (0.7)	1.32 (0.79–2.19)	42	0.51 (0.13–2.09)	3

Group	Description	Mutation Types Included	NMD	Protein	No. (%) With Mutation	Breast Cancer, HR (95% CI)	No. of Women With Breast Cancer	Ovarian Cancer, HR (95% CI)	No. of Women With Ovarian Cancer
5	Founder mutation c.5946delT				1341 (11.3)	0.79 (0.60–1.03)	579	0.77 (0.46–1.32)	155

Abbreviations: FS, frame shift; GR, genomic rearrangement; HR, hazard ratio; IF, in-frame; NE, not estimable; NMD, nonsense-mediated decay; NS, nonsense; MS, missense; OF, out of frame; SP, splicing.

Table 3

Mutation-Specific Risk Groups: Ages at Diagnosis of Breast Cancer or Ovarian Cancer

Group[a]	No. of Women With Breast Cancer	No. of Women With Ovarian Cancer	Mean Age at Diagnosis, y					
			Breast Cancer			Ovarian Cancer		
			With Mutation	Without Mutation	FDR *P* Value	With Mutation	Without Mutation	FDR *P* Value
BRCA1 **(n = 19 581)**								
a	796	336	40.4	42.4	<.001	50.2	52.5	<.001
1	5469	2038	41.2	40.1	<.001	50.8	50.3	.32
2	7318	2524	41.2	40.4	.001	51.2	50.2	.06
3	250	74	40.7	39.4	.07	50.5	52.2	.29
4	2986	781	40.5	41.1	.006	50.6	50.0	.32
5	2698	850	40.2	41.1	<.001	50.27	51.11	.03
5a	1033	391	40.43	42.41	<.001	50.22	52.49	<.001
5b	1665	459	40.5	41.5	<.001	50.6	50.0	.34
6	899	241	40.6	49.8	.60	50.5	49.8	.59
6a	681	171	40.6	41.1	.39	50.6	49.3	.32
6b	202	64	40.7	40.2	.60	50.5	51.2	.59
7	925	249	40.6	40.9	.60	50.6	49.8	.34
8	26	8	40.6	41.8	.60	50.5	48.4	.59
9	2000	520	40.5	41.1	.02	50.6	50.1	.34
BRCA2 **(n = 11 900)**								
a	534	155	43.3	45.8	<.001	56.5	56.9	.83
1	5383	803	44.5	43.4	.008	56.5	56.5	.93

Group[a]	No. of Women With Breast Cancer	No. of Women With Ovarian Cancer	Mean Age at Diagnosis, y					
			Breast Cancer			Ovarian Cancer		
			With Mutation	Without Mutation	FDR *P* Value	With Mutation	Without Mutation	FDR *P* Value
2	118	12	42.4	43.6	.42	55.7	55.6	.94
3	76	9	42.4	43.6	.42	56.3	56.5	.94
4	42	3	42.4	43.6	.50	53.0	56.5	.94
5	579	155	43.33	45.83	<.001	56.47	56.88	.65

Abbreviations: FDR, false discovery rate.

[a]Group descriptions appear in Table 2.

Table 4

Risks Associated With Specific Binding Domains: Comparison of Mutations Not in the Domain (ie, the Reference Group) vs Those Within the Domain

				Breast Cancer			Ovarian Cancer		
Gene	Domain	Binding Partner	Region	No. With/Without Mutation[a]	HR (95% CI)	FDR *P* Value	No. With/Without Mutation[a]	HR (95% CI)	FDR *P* Value
BRCA1	RING	BARD1	c.72–192	781/595	1.13 (1.02–1.26)[b,c]	.04	205/1171	0.81 (0.67–0.97)	.07
	Coiled coil	PALB2	c.3759–3819 or c.4191–4272	122/90	1.20 (0.93–1.54)[d]	.16	39/173	0.97 (0.62–1.50)	.88
	BRCT	BACH1	c.4926–5169 or c.5268–5526	1203/832	1.26 (1.15–1.38)[e,f]	<.001	298/1737	0.86 (0.74–1.01)	.09
BRCA2	BRC	RAD51	c.3006–3108, c.3636–3738, c.4263–4365, c.4551–4653, c.4992–5094, c.5511–5613, c.5913–6015, or c.6153–6255	810/992	0.67 (0.56–0.79)[g,h]	<.001	205/1597	1.09 (0.78–1.53)	.77
	DNA binding		c.7437–8001	464/336	1.17 (0.99–1.38)	.08	63/737	1.06 (0.71–1.59)	.77
	OB folds	ssDNA	c.8010–8400 or c.9156–9570	512/364	1.18 (1.01–1.37)[h,i]	.07	50/826	0.57 (0.39–0.84)	.02
	Tower	RAD51	c.8443–8616	193/154	1.20 (0.92–1.56)	.19	18/329	0.42 (0.18–1.00)[j]	.10

Abbreviations: FDR, false discovery rate; HR, hazard ratio; NMD, nonsense-mediated decay; OB, oligonucleotide-binding.

[a] Among 19 581 carriers of *BRCA1* mutations and 11 900 carriers of *BRCA2* mutations.

[b] Missense mutations only: HR = 1.42; 95% CI, 1.06–1.90.

[c] Mutations conferring NMD only: HR = 2.56; 95% CI, 1.03–6.34.

[d] Mutations conferring NMD only: HR = 1.35; 95% CI, 1.05–1.72.

[e] Premature termination codon mutations only: HR = 1.31; 95% CI, 1.17–1.47.

[f] Mutations conferring NMD only: HR = 1.38; 95% CI, 1.20–1.59.

[g] Premature termination codon mutations only contributed to this estimate.

[h] Mutations conferring NMD only: HR = 1.26; 95% CI, 1.07–1.48.

[i] Premature termination codon mutations only: HR = 1.26; 95% CI, 1.07–1.48.

[j] Mutations conferring NMD only: HR = 0.31; 95% CI, 0.13–0.77.

Table 5

Representative Cancer Penetrances by Age 70 Years: Baseline Risk and Modified Risk in Mutation Group

Gene	Cancer Site	Statistically Significant Mutation-Specific Relative Risk	Mutation Groups Corresponding to These Relative Risks, Tables 2–3 (Group No.)	Mutation Prevalence, %[a] (EMBRACE Data)	Overall Penetrance to Age 70 Years, % (BOADICEA)	Mutation-Specific Penetrance to Age 70 Years, % (95% CI)
BRCA1	Breast	1.4	All founder mutations (5)	16	59	69 (56–83)
	Ovary	0.7	All founder mutations (5)	16	34	26 (10–43)
BRCA2	Breast	0.7	Truncating mutations within the BRC domains (Table 3)	11	51	40 (27–54)
	Ovary	0.3	Not PTC (2)	0.6	11	3 (0–38)

[a]Defined as the proportion of heterozygous mutation carriers with this mutation class with the specified cancer and *BRCA1* or *BRCA2* mutation in the EMBRACE data set.